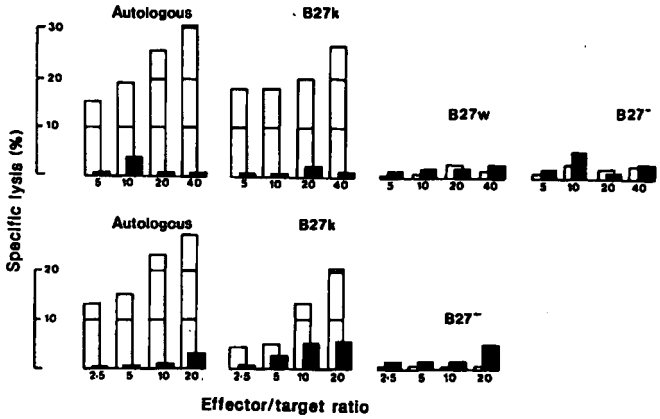

Fig. 2 Recognition of virally infected cells is restricted by the HLA-B27 subtype in the context of which was immunized. Immunization of mice, preparation of targets, and CTL assays were carried out as described in the legend to Fig. 1. In this experiment CTL were tested on infected (open bars) and uninfected (solid bars) targets only, having established that such CTL are virus-specific (Fig. 1). Bars indicate percentage of specific lysis of target cells by the given effector to target ratio measured in triplicate. The top panels depict the response of anti-influenza CTL on autologous, HLA-B27K-matched or mismatched targets, as indicated. The bottom panels show the results for the anti-Sendai response on autologous, HLA-B27K-matched, and HLA-B27 negative targets. The HLA-types of the targets were as described in the legend to Fig. 1.

ability of T cells to discriminate between epitopes on other 'foreign' antigens such as viral glycoproteins. In the experiments reported here, introduction into the germ line of all the information necessary to generate human HLA-B27 antigens has resulted in tolerance and has allowed us to test whether HLA-B27, next to the mouse's own class I MHC molecules, might serve as a restriction element for murine cytotoxic T cells. Our results show that this is indeed the case. It should be emphasized that authentic human HLA antigens are generated only when the genes encoding both human subunits are present, in contrast with the hybrid antigens produced if only HLA heavy-chain genes are used to construct transgenic animals[21]. In the latter case, a direct comparison of cells from transgenic mice with human cells as either targets or effectors should take into account the fact that human and mouse β_2m show only 70% similarity, and that hybrid molecules are distinct, at least serologically, from the parental molecules[22,23].

Our results strongly suggest that selection of the T-cell receptor repertoire is not appreciably affected by conserved regions of the MHC class-I molecule that contain 'species-specific' residues (for example the α-3 domain, or β_2m). The more conserved areas may merely serve to position the antigen-presenting segments of class-I antigens in the correct configuration in relation to the T-cell receptor. It is more likely that indeed the most variable regions of the class-I molecule are directly involved. Although some species-specific residues may be identified in these regions, they obviously still allow the selection of such T-cell clones that can use HLA-B27 as a restriction element.

In humans, several diseases show a strong association with the HLA-B27 antigen. At present, it cannot be decided whether this association is due to holes in the repertoire, resulting from the need for tolerance to HLA-B27 in HLA-B27 positive individuals, or alternatively, by unique properties of HLA-B27 as an antigen presenting element. The experiments reported here are a first step towards the validation of HLA-B27 transgenic mice as a possible tool in the study of HLA-disease associations.

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Cytotoxic T lymphocytes against a soluble protein

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Thymus-derived (T) lymphocytes recognize antigen in conjunction with surface glycoproteins encoded by major histocompatibility complex (MHC) genes[1–3]. Whereas fragments of soluble antigens are presented to T helper lymphocytes (T_H), which carry the CD4 antigen, in association with class II MHC molecules[4], CD8-bearing cytotoxic T lymphocytes (CTL) usually see cellular antigens (for instance virally-encoded proteins) in conjunction with MHC class I molecules[5,6]. The different modes of antigen presentation may result from separate intracellular transport: vesicles containing class II molecules are thought to fuse with those carrying endocytosed soluble proteins. Class I molecules, in contrast, can only pick up degradation products of intracellular proteins (see refs 7 and 8). This makes biological sense; during an attack of a virus, class I-restricted CTL destroy infected cells and class II-restricted T_H guide the humoural response to neutralize virus particles and toxins. But here we provide evidence that CTL specific for ovalbumin fragments can be induced with soluble protein, and that intracellular protein degradation provides epitopes recognized by these CTL. These findings suggest the existence of an antigen presenting cell that takes up soluble material and induces CTL.

It is well documented that fragments of cytoplasmic and membrane-associated proteins, such as influenza nucleoprotein and MHC molecules, can be seen in the context of class I molecules[5,9–11]. But the possibility that antigens taken up from outside cells can be presented to class I-restricted T lymphocytes

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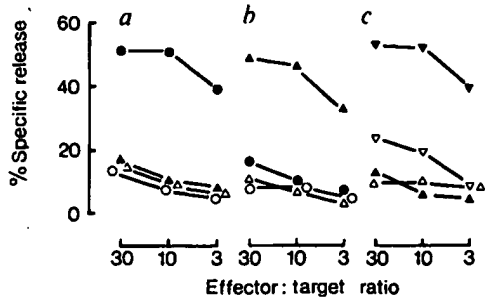

Fig. 1 Specificity of secondary *in vitro* cultures after *in vivo* immunization with soluble ovalbumin. Cultures derived from C57BL/6 (*a*, *c*) or DBA/2 (*b*) mice that had been immunized with ovalbumin in the presence (*a*, *b*) or absence (*c*) of syngeneic feeders were tested for their lytic activity on EL4 (C57BL/6, ●○▼▽) and P815 (DBA/2, ▲△) tumour targets in the presence (solid symbols) or absence (open symbols) of ovalbumin fragmented by trypsin digestion (*a*, *b*) or treated with CNBr (*c*).
Methods. Six-week-old C57BL/6 and DBA/2 (IFFA Credo) mice were intravenously injected either with a suspension of 2×10^6 syngeneic irradiated (2,000 rad) spleen cells in 0.5 ml of a solution of 4 mg ml^{-1} ovalbumin (Sigma) phosphate-buffered saline (PBS) after 30 min incubation on ice or with 0.5 ml 0.2 mg ml^{-1} ovalbumin in PBS. After 7 days animals were killed, spleens removed and cultured in single-cell suspensions in flat-bottomed 24-well plates (Costar) at 1×10^7 cells ml^{-1} in IMDM (Gibco) supplemented with 10% fetal calf serum (Boehringer), 2 mM glutamine, non-essential amino acids, 1 mM hydroxypyruvate, penicillin at 100 international units ml^{-1}, streptomycin at 100 μg ml^{-1}, gentamycin at 50 μg ml^{-1} (Sigma). Spleen cells obtained from animals immunized with feeders were grown in the presence of soluble ovalbumin at 0.7 mg ml^{-1}, to the other cultures CNBr-fragmented ovalbumin was added at 0.1 mg ml^{-1}. Ovalbumin was digested by trypsin (Sigma) as described previously[16] and fragmented by CNBr (Sigma) according to ref. 17 (tOva and cOva, respectively). Both preparations were checked for complete degradation by gel filtration. For the CML assay, ^{51}Cr-labelled target cells were preincubated with or without fragmented ovalbumin at 0.3 mg ml^{-1} in supplemented IMDM for 60 min at 37 °C. For the actual test 1×10^4 target cells suspended in the same medium were incubated for 4½ h in round-bottomed microtitre plates (total volume, 0.2 ml; Costar) at effector to target ratios ranging from 30:1 to 1:1. Per cent specific lysis of these target cells was calculated as described elsewhere[5].

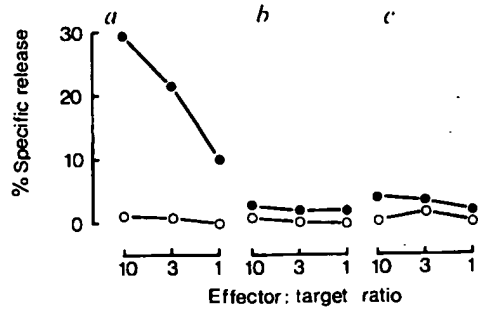

Fig. 2 Specificity of a cloned cytotoxic T lymphocyte line specific for CNBr-fragmented ovalbumin. The killing activity of C11 was tested against EL4 (*a*), P815 (*b*) and S.AKR (AKR/J, *c*) in the presence (●) and absence(○) of CNBr-fragmented ovalbumin.
Methods. The CTL clone C11 was isolated by limiting dilution from a secondary culture of spleen cells of a C57BL/6 mouse immunized with ovalbumin, and maintained by weekly subculture with 0.1 mg ml^{-1} CNBr-fragmented ovalbumin and irradiated syngeneic spleen cells in supplemented IMDM plus an exogenous source of IL-2. The CML assay was performed as for Fig. 1.

is suggested by several experimental lines of evidence. For instance, female mice reject syngeneic male skin-grafts after priming with male cells of another haplotype, and cytotoxic T lymphocytes of a given H-2 (mouse MHC) haplotype can be immunized against an array of minor and other antigens carried on cells of another haplotype[12,13]. Therefore, it was proposed that foreign antigens arising from the cellular debris are taken up by host cells and presented in conjunction with class I MHC[8].

We immunized mice with soluble ovalbumin, previously defined as a class II-restricted antigen. Irradiated syngeneic spleen cells incubated with a solution of ovalbumin were injected intravenously. Seven days later spleen cells were cultured in the presence of undigested ovalbumin for 5 days. Alternatively, soluble ovalbumin was only used for *in vivo* priming, and the spleen cells were grown *in vitro* together with CNBr-fragmented ovalbumin (cOva). To test for activity restricted by MHC class I molecules, tumour targets that do not express class II molecules were used (EL4, P815 and S.AKR). As demonstrated in Fig. 1, neither immunization protocol resulted in appreciably lytic activity towards allogeneic targets in the presence or absence of ovalbumin preparations. But C57BL/6 (H-2^b) and DBA/2 (H-2^d) mice immunized with ovalbumin mounted a specific response to the syngeneic targets, EL4 (H-2^b) or P815 (H-2^d) respectively, in the presence of their specific antigen, whereas in the absence of antigen only minor background activity could be observed. Preliminary experiments showed that in some cases minor but significant reactivity against ovalbumin could be obtained without prior *in vivo* priming (data not shown) suggesting that this 'background' reactivity might actually be directed against a component of the culture medium that can be presented by the target cell. The presence of syngeneic feeder cells at the time of immunization had no effect. Fragments of other proteins, for example pigeon cytochrome *c* or hen egg lysozyme (HEL), could not substitute for cOva or tOva. But CNBr-fragmented HEL could reveal specific MHC class I-restricted lytic activity after immunization with the soluble protein. Similar results could be obtained with blast cells as targets excluding any peculiar properties of the tumour cells (data not shown).

To further characterize the cell population responsible for this reactivity we established a cloned T-lymphocyte line, C11 (C57BL/6 derived) from a secondary *in vitro* culture directed against cOva. As determined by indirect surface immunofluorescence, C11 has the phenotype of a conventional CTL: CD1$^+$, CD4$^-$, CD8$^+$ (data not shown). C11 had a restriction pattern identical to the uncloned population (Fig. 2). The syngeneic target EL4 was susceptible to lysis only in the presence of cOva, otherwise it was not killed by C11, nor were allogeneic targets. These findings indicate that the induced MHC class I-restricted response can be carried by conventional CD8$^+$ CTL.

We wanted to examine whether this immune response against ovalbumin is directed against epitopes on this molecule that were expressed not only by chemical and enzymatic fragmentation, but also by intracellular protein degradation within the target cell. EL4 cells were transfected with BOVAneo expressing ovalbumin cDNA. The cell line EL4.Ova4-17 was subcloned and tested for susceptibility for lysis mediated by C11 (Fig. 3). The extent of lysis of the parental cell line in the presence of cOva (Fig. 3*a*) is comparable to that of the transfected cell line (Fig. 3*b*). In contrast, C11 could not kill EL4 grown in medium containing soluble undigested ovalbumin, excluding the possible explanation that secreted ovalbumin was taken up by EL4 and then presented in conjunction with MHC. It is noteworthy, however, that trypsin digestion of ovalbumin seems to destroy the epitope recognized by C11.

Although it cannot formally be excluded that ovalbumin is fragmented outside cells during the *in vivo* immunization, this explanation seems rather unlikely in view of findings that immunization with tOva, in all cases, failed to induce a measurable response (data not shown), whereas immunization with undigested ovalbumin always resulted in responses to epitopes

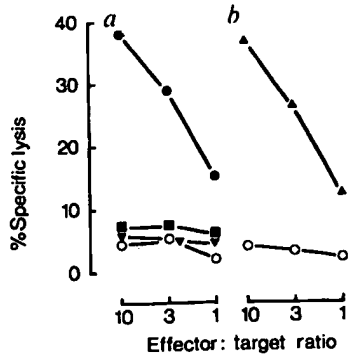

Fig. 3 Ovalbumin-transfected target cells can be lysed by the cloned CTL C11. The susceptibility to lysis of the parental line EL4 (○, ●, ■, ▼) in the presence of CNBr-fragmented (●), trypsin-digested (■) whole ovalbumin (▽) or uncoated (○) was compared to the ovalbumin transfected line EL4.Ova4-17 (△). **Methods.** Ovalbumin cDNA[18] was introduced into the BMIG*neo* expression vectors (H.K. & F. Melchers, in preparation) and EL4 cells were transfected with the resulting construct (BOVA *neo*). Neomycin-resistant EL4 clones were tested for ovalbumin expression by intracellular indirect immunofluorescence with a monoclonal mouse anti-ovalbumin antibody provided by D. Gray (data not shown). The line EL4.Ova4-17 was picked, subcloned by limiting dilution and tested for susceptibility for lysis mediated by C11. To test for presentation of undigested ovalbumin EL4 was grown in medium containing 10 mg ml^{-1} ovalbumin for 18 h.

exposed by trypsin digestion. As it is clear from our data that at least part of the activity induced by our immunization protocols is carried by conventional CTL recognizing epitopes found on ovalbumin, there cannot be a complete separation of the two pathways of antigen presentation for MHC class I-restricted and MHC class II-restricted antigens. Our data indicate the existence of a mechanism allowing exogenous antigens to be presented in conjunction with MHC class I molecules in a similar way to antigens expressed within the cell. The fact that EL4 grown in soluble ovalbumin cannot be lysed by C11, in contrast to its counterpart transfected with the ovalbumin gene, points to the existence of a specialized antigen-presenting cell responsible for the induction of CTL. But, in contrast to previous proposals[8], this antigen-presenting cell can take up soluble material. It should be of great interest to define the mechanisms and cell populations involved in the induction of CTL against soluble antigens further, as these cells might interfere with the immune response in the sense of a suppressive system.

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Loss of heterozygosity of chromosome 3p markers in small-cell lung cancer

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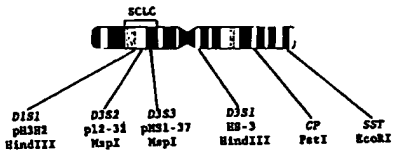
Specific chromosomal deletions sometimes associated with tumours such as retinoblastoma (chromosome 13q14)[1] and Wilm's tumour (chromosome 11p13)[2] have led to the hypothesis that recessive genes may be involved in tumorigenesis[3]. This hypothesis is supported by demonstration of allele loss specific for these regions using polymorphic DNA markers[4–9] and by the isolation of a complementary DNA clone for the retinoblastoma gene[10]. A cytogenetic deletion in chromosome 3 (p14–p23) was reported in small-cell lung cancer (SCLC) by Whang-Peng *et al*[11,12]. At least one homologue of chromosome 3 was affected in the majority of SCLC tumours; however, the multiple chromosomal changes seen presented the possibility that chromosome 3 was rearranged, not deleted. We used polymorphic DNA probes for chromosome 3p and compared tumour and constitutional genotypes of nine SCLC patients. Our data show loss of alleles of chromosome 3p markers in tumour DNA of all nine patients supporting the hypothesis that this region contributes to tumorigenesis in SCLC.

DNA samples prepared from tumour and normal tissues of the same individual were obtained from cultured cell lines H128

Table 1 Chromosome 3 markers in SCLC



Group 1:						
H209BL	1,2	1,2	1,2	1,1	-	1,2
H209	1	1	1	1,1	-	1,2
N-1	1,2	1,1	1,1	1,2	1,2	1,1
SCLC-1	2	1,1	1,1	1,2	1,2	1,1
N-2	1,2	1,2	1,1	1,2	1,2	1,2
SCLC-2	2	2	1,1	1,2	1,2	1,2
N-3	1,2	1,1	1,2	1,2	1,2	1,1
SCLC-3	2	1	1	1,2	1,2	1,1
N-5	1,2	1,2	1,2	1,1	1,2	1,2
SCLC-5	2	1	1	1,1	1,2	1,2
N-6	2,2	1,2	1,2	1,2	1,1	1,2
SCLC-6	2	1	2	1,2	1,1	1,2
N-7	1,1	1,2	1,1	1,2	1,1	1,1
SCLC-7	1	2	1,1	1,2	1,1	1,1
Group 2:						
H128BL	2,2	1,2	1,2	1,2	-	1,1
H128	2	1	1	2	-	1
N-4	1,2	1,2	1,2	1,2	1,1	1,1
SCLC-4	1	2	1	1	1	1
Other lung cancers:						
N	2,2	1,2	1,1	1,2	2,2	1,1
Large cell	2,2	1,2	1,1	1,2	2,2	1,1
N	1,2	2,2	1,1	1,1	1,2	1,1
Adenocarcinoma	1,2	2,2	1,1	1,1	1,2	1,1

The numbers 1 and 2 refer to the alleles of a given locus. The copy number of homozygous markers is not known in the tumour samples. A dash indicates that the sample was not scored for a given polymorphism. Transferrin and somatostatin were not informative with *Eco*RI and *Bam*HI, respectively, for any of these individuals nor was pH3E4 for *Hin*dIII digests. Locus *D1S1* is now called *DNF15S1*.